

The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

1. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: ~~D405~~, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
2. (Original) The enzyme mixture of claim 1, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.
3. (Currently Amended) The enzyme mixture of claim 2, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), Pwo DNA polymerase ~~Deep Vent DNA polymerase~~, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase (SEQ ID NO. 10), PGB-D DNA polymerase (Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.
- 4-9. (Cancelled)
10. (Currently Amended) The enzyme mixture of claim 1 ~~or 9~~, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: ~~D405E~~, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
11. (Previously Presented) The enzyme mixture of claim 1, further comprising a PCR enhancing factor and/or an additive.
12. (Currently Amended) A kit comprising a first enzyme, a second enzyme, and packaging material therefor, wherein said first enzyme comprises a DNA polymerization activity, said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein

said mutation(s) are selected from amino acid positions selected from the group consisting of: ~~D405~~, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

13. (Original) The kit of claim 12, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

14. (Currently Amended) The kit of claim 13, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), ~~Pwo Deep Vent~~ DNA polymerase, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase (SEQ ID NO. 10), PGB-D DNA polymerase(Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.

15-19. (Cancelled)

20. (Currently Amended) The kit of claim 12, ~~or 18~~, further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.

21. (Cancelled)

22. (Currently Amended) The kit of claim 12 ~~or 21~~, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: ~~D405E~~, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

23. (Withdrawn) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

24. (Withdrawn) The method of claim 23, wherein said nucleic acid template is a DNA [or an RNA] molecule.

25. (Withdrawn) The method of claim 24, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

26. (Withdrawn) The method of claim 25, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

27-29. (Cancelled)

30. (Withdrawn) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

31. (Withdrawn) A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

32. (Withdrawn) The method of claim [28, ]30, or 31, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

33. (Withdrawn) The method of claim 23 [32], wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
34. (Withdrawn) The method of claim 23, 30 or 31, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.
35. (Withdrawn) The method of claim 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
36. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: ~~D405~~, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
37. (Currently Amended) The enzyme mixture of claim 36, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: ~~D405E~~, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
38. (Previously Presented) The enzyme mixture of claim 36, wherein said mutant Pfu DNA polymerase comprises a mutation at amino acid position G387.
39. (Previously Presented) The enzyme mixture of claim 36, wherein said mutant Pfu DNA polymerase comprises a mutation of G387P.
40. (Previously Presented) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a KOD DNA polymerase, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

41. (Previously Presented) The enzyme mixture of claim 40, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

42. (Previously Presented) The enzyme mixture of claim 40, wherein said mutant Pfu DNA polymerase comprises a mutation at amino acid position G387.

43. (Previously Presented) The enzyme mixture of claim 40, wherein said mutant Pfu DNA polymerase comprises a mutation of G387P.

44. (Currently Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase (SEQ ID NO. 10), and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

45. (Previously Presented) The enzyme mixture of claim 44, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

46. (Previously Presented) The enzyme mixture of claim 44, wherein said mutant Pfu DNA polymerase comprises a mutation at amino acid position G387.

47. (Previously Presented) The enzyme mixture of claim 44, wherein said mutant Pfu DNA polymerase comprises a mutation of G387P.

48. (Currently Amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: ~~D405~~, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor.

49. (Previously Presented) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a KOD DNA polymerase, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor.

50. (Currently Amended) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase (SEQ ID NO. 10), and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, and packaging material therefor.

51. (Previously Presented) The kit of claim 48, 49, or 50, wherein said kit further comprises a reagent selected from the group consisting of: dNTPs, reaction buffer, primer, and DNA enhancing factor.